Significant Association of Ku80 Single Nucleotide Polymorphisms with Colorectal Cancer Susceptibility in Central Taiwan

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Abstract. *Aim: To evaluate the association between the polymorphisms of the Ku80 gene and the risk of colorectal cancer in Central Taiwan. Materials and Methods: In this hospital-based case-control study, the association of Ku80 G-1401T rs828907, Ku80 C-319T rs11685387 and Ku80 intron 19 rs9288518 polymorphisms with colorectal cancer risk in a central Taiwanese population was investigated. In total, 362 patients with colorectal cancer and 362 age- and gender-matched healthy controls recruited from the China Medical Hospital in central Taiwan were genotyped. Results: A significantly different distribution was found in the frequency of the Ku80 G-1401T genotype, but not the Ku80 C-319T or intron 19 genotypes, between the colorectal cancer and control groups. The T allele Ku80 G-1401T conferred a significantly (p=0.0069) increased risk of colorectal cancer. As for Ku80 C-319T and intron 19 polymorphisms, there was no difference in distribution between the colorectal cancer and control groups. Gene interactions with smoking, but not with alcohol consumption, were significant for Ku80 G-1401T polymorphism. The Ku80 G-1401T GT and TT genotype in association with smoking conferred an increased risk of 2.537 (95% confidence interval=1.398-4.601) for colorectal cancer. Conclusion: These results provide the first evidence that the T allele of the Ku80 G-1401T may be associated with the development of colorectal cancer and may be a novel useful marker for primary prevention and anticancer intervention.*

The incidence and age-adjusted mortality of colorectal cancer (CRC) has drastically increased in the past 2 decades in Taiwan. In 2008, the incidence and mortality of CRC took the third place among the common types of cancer. Etiological studies have attributed more than 85% of CRC to environmental factors (1, 2), and in particular meat consumption, cigarette smoking, and exposure to carcinogenic aromatic amines, such as arylamines and heterocyclic amines (3-5). These carcinogens are thought of as DNA damage inducers and promote various types of DNA adducts, such as DNA base damage, DNA single-strand breaks and double-strand breaks (DSBs) (6). The DSBs may lead to dramatic genome instability, which is closely related to carcinogenesis (7, 8). There are two specific DNA repair pathways responsible for DSBs repairing, homologous recombination (HR) repair and the non-homologous end-joining (NHEJ) (7). Most of the DSBs are repaired by NHEJ with the involvement of several key components (9). Once DSBs happen in genomic DNA, they are first recognized by a heterodimeric DNA-binding component KU, which is formed from Ku70 and Ku80 (10). The *Ku80* gene is located on the 2q35 of chromosome and has 21 exons (11). Former studies have indicated that mutation of *Ku80* may affect the age at cancer onset (12).

Mounting single nucleotide polymorphisms (SNPs) have been confirmed as genetic factors associated with cancer (13-18). Recently, the *Ku80* gene has been reported to play a role in cancer development, but the association of its SNPs with CRC has not been investigated yet. In this study, the role of *Ku80* in central Taiwanese population was investigated by examining SNPs of *Ku80* in CRC.

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Materials and Methods

Study population and sample collection. The study population consisted of 362 case patients and 362 cancer-free control volunteers. Three hundred and sixty-two cancer patients diagnosed with CRC were recruited at the outpatient clinics of general surgery between 2002-2008 at the China Medical University Hospital, Taichung, Taiwan, Republic of China. The clinical characteristics of patients, including histological details, were all graded and defined by expert surgeons (Dr. Yang's team). All patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. An equal number of non-cancer healthy volunteers were selected as controls by matching for age, gender and some indulgences after initial random sampling from the Health Examination Cohort of the hospital. The exclusion criteria of the control group included previous malignancy, metastasized cancer from other or unknown origin, and any familial or genetic diseases. This study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consent was obtained from all participants.

Genotyping assays. Genomic DNA was prepared from peripheral blood leucocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to previous papers (19, 20). Briefly, the following primers were used for *Ku80* G-1401T rs828907: 5'-TAGCTGACAACCTCACAGAT-3' and 5'-ATTCAGAGGTGCT CATAGAG-3'; for *Ku80* C-319T rs11685387: 5'-TCTAACTCCAGA GCTCTGAC-3' and 5'-AACTCTGAGCATGCGCAGAT-3'; and for *Ku80* intron 19 rs9288518: 5'-GGTGTGAAGACCTATCAATC-3' and 5'-TTACAGAACAAGCCTTGCAC-3'. The following cycling conditions were performed: one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 s, 55˚C for 30 s and 72˚C for 30 s; and a final extension at 72˚C for 10 min. The PCR products were studied after digestion with BfaI, SpeI and BsrI restriction enzymes for *Ku80* G-1401T rs828907 (cut from 252 bp G type into 81+171 bp T type), *Ku80* C-319T rs11685387 (cut from 311 bp C type into 108+203 bp T type) and *Ku80* intron 19 rs9288518 (cut from 275 bp A type into 110+165 bp G type), respectively.

Statistical analyses. Only those matches with all SNP data were selected for final analyses. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotype frequencies of *Ku80* SNPs in the control subjects from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's χ^2 test or Fisher's exact test (when the expected number in any cell was less than five) was used to compare the distribution of the *Ku80* genotypes between cases and controls. Data was recognized as significant when the statistical p was less than 0.05.

Results

The frequency distributions of selected characteristics of CRC patients and controls are shown in Table I. These characteristics of patients and controls are all well matched. None of these differences between groups were statistically significant (p>0.05) (Table I).

The frequency distributions of the genotypes for *Ku80* G-1401T, C-319T and intron 19 in controls and CRC patients are shown in Table II. The genotype distribution of the genetic polymorphisms of Ku80 G-1401T was significantly different between CRC and control groups (p=0.0069), while those for C-319T and intron 19 were not significant (p>0.05) (Table II). To sum up, the Ku80 G-1401T heterozygous was significantly associated with CRC susceptibility.

The frequency distributions of the alleles for *Ku80* G-1401T, C-319T and intron 19 in controls and CRC patients are shown in Table III. The distributions of all these polymorphisms were in Hardy-Weinberg equilibrium and were similar between controls and CRC patients. The T allele of the *Ku80* G-1401T polymorphism was significantly associated with CRC (p=0.0001) (Table III).

The genotype distribution of various genetic polymorphisms of *Ku80* G-1401T was significantly different between CRC and control groups who had a smoking habit (p=0.003) (Table IV), while those for C-319T and intron 19 were not significant (p>0.05). The T allele frequency was significantly higher in CRC patients who smoked than in non-cancer controls and patients who did not smoke. In central Taiwan, individuals with *Ku80* G-1401T GT or TT who smoked were approximately 2.5-fold more likely to have CRC than those who did not smoke (Table IV).

Table I. *Frequency distributions of characteristics among colorectal cancer patients and controls.*

Characteristics	Patients (n=362)			Controls (n=362)			p-Value
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			64.4 (6.2)			63.8 (5.8)	0.149
Age group (years)							0.932
≤60	93	25.7%		95	26.2%		
>60	269	74.3%		267	73.8%		
Gender							0.707
Male	209	56.1%		203	57.7%		
Female	153	43.9%		159	42.3%		
Habits							
Cigarette smokers	84	23.2%		91	25.1%		0.602
Alcohol drinkers	51	14.1%		44	12.2%		0.509

Discussion

In the present study, three polymorphisms of the *Ku80* gene, *Ku80* G-1401T, C-319T and intron 19, were selected to evaluate their associations with CRC risk in central Taiwan. This is the first study which focuses on the association between the polymorphisms of *Ku80* and CRC susceptibility. It was found that only the *Ku80* G-1401T polymorphism has a statistical significance in association with increased CRC, while the *Ku80* C-319T and *Ku80* intron 19 genotypes have no effect (Tables II and III). In the population with a smoking habit, the genetic effect of the *Ku80* G-1401T on CRC risk is

Table II. *Distribution of Ku80 genotypes among colorectal cancer patients and controls.*

Genotype	Controls	%	Patients	%	*p*-Value[a]
G-1401T rs828907					**0.0069**
GG	272	75.1%	233	64.4%	
GT	63	17.4%	91	25.1%	
TT	27	7.5%	38	10.5%	
C-319T rs11685387					0.4522
CC	49	13.5%	40	11.0%	
CT	85	23.5%	96	26.5%	
TT	228	63.0%	226	62.4%	
Intron 19 rs9288518					0.3403
AA	35	9.6%	40	11.0%	
AG	123	34.0%	105	29.0%	
GG	204	56.4%	217	60.0%	

[a]*p* based on χ^2 test.

Table III. *Distribution of Ku80 alleles among colorectal cancer patients and controls.*

Allele	Controls	%	Patients	%	*p*-Value[a]
G-1401T rs828907					**0.0010**
Allele G	607	83.8%	557	76.9%	
Allele T	117	16.2%	167	23.1%	
C-319T rs11685387					0.7150
Allele C	183	25.3%	176	24.3%	
Allele T	541	74.7%	548	75.7%	
Intron 19 rs9288518					0.6750
Allele A	193	26.7%	185	25.6%	
Allele G	531	73.3%	539	74.4%	

[a]*p* based on χ^2 test.

Table IV. *Ku80 G-1401T genotype and colorectal cancer after stratification by cigarette smoking.*

Variables	Ku80 G-1401T genotype			
	GG	GT+TT	*p*-Value[a]	OR (95% CI)[b]
Smokers			**0.0030**	
Controls	47	36		1.00
Patients	35	68		**2.537 (1.398-4.601)**[c]
Non-smokers			0.280	
Controls	225	54		1.00
Patients	198	61		1.284 (0.849-1.940)

[a]*p* based on χ^2 test; [b]ORs were estimated with multivariate logistic regression analysis; [c]Statistically identified as significant.

much more significant. In the smoking groups, the T allele can obviously raise the CRC risk (Table IV). There was no significant joint effect between Ku80 G-1401T and alcohol drinking on CRC risk (data not shown). According to these findings, it was proposed that the T allele of polymorphism *Ku80* G-1401T may play a role in carcinogenesis. Non smokers carrying the T allele may have a similar efficiency in removing DSBs to those with T allel smokers, but in DNA damage is increased significantly, thus those with the T allele may not have enough capacity to remove all the DSBs promptly and efficiently, thus increasing their CRC risk.

The limitation of this hospital-based case-control study is that the results might not be generalized to populations in Taiwan overall. However, the records of the risk factors, smoking and alcohol drinking habits, and the genotyping methods are reliable to minimize any possible bias. Analysis of some diet habits, such as meat, vegetable/fruit and fish/shrimp consumption, cannot be performed due to a shortage of data about these items from the questionnaires between 2002-2005, which have been much improved since then (21). In this study, a novel potential biomarker of CRC, *Ku80* G-1401T, was found together with proof of the importance of smoking in CRC. Carcinogenesis is indeed a complex and multistep process, and it is difficult to reveal the causes of CRC with simply one hypothesis. Thus, the findings in this paper can only reveal part of the process of colorectal carcinogenesis. Functional studies on the polymorphic variants and further studies on other genotypic variants involved in DSBs and other repair pathways are being conducted and warranted all over the world (22, 23). The continuous enlargement of the investigation population is also paramount.

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